Poly(lactide-*co*-glycolide) Decomposition Kinetics in Vivo and in Vitro

Richard A. Kenley,* Maryann Ott Lee, T. Randolph Mahoney, II,† and Lynda M. Sanders

Syntex Research, 3401 Hillview, Palo Alto, California 94304. *Received October 16, 1986*

ABSTRACT: We examined ten *d,l*-lactide/glycolide copolymers representing a range of monomer ratios and molecular weights. We characterized the molecular weight distributions by intrinsic viscosity measurements (in two solvents) and by size-exclusion chromatography (SEC) using the "universal calibration" procedure. In tetrahydrofuran, the copolymers obey the following Mark–Houwink relationship: $[\eta]_{THF} = (1.07 \times 10^{-4})M^{0.761}$. In hexafluoro-2-propanol they obey $[\eta]_{HFIP} = (1.67 \times 10^{-4})M^{0.794}$. Using a 50:50 lactide:glycolide copolymer, we prepared cylindrical samples and incubated them for timed intervals in pH 4.5–7.4 aqueous buffers at 37 °C. We also implanted parallel samples subcutaneously in rats. We then monitored the time-dependent changes in sample total weight (TW) and molecular weight (MW). TW and MW profiles were superimposable for all samples, demonstrating pH-independent hydrolysis in vitro and equivalent in vivo versus in vitro copolymer degradation rates. TW loss lagged behind MW loss, indicating copolymer erosion via internal (versus surface) hydrolysis and dissolution. The copolymer MW loss rates adhered well to a pseudo-first-order kinetic model where the number of chain cleavages (X) per initial number average molecule is given by the following expression: $\ln [X] = -2.04 - 1.08t(\text{week}^{-1})$.

Introduction

Polymers find increasing application in the pharmaceutical industry as matrices for "controlled-release" drug products. This currently active subdiscipline has been extensively reviewed.[1-10] One approach to these advanced pharmaceuticals involves polymers that degrade in vivo following (or concurrently with) drug release, thereby influencing the release profile. Degradable polymers also convert the matrix to soluble fragments that can be metabolized or eliminated. Among the various polymer types considered as degradable carriers for drugs, hydrolytically labile polyesters have evoked considerable interest. In particular, lactide and glycolide homo- and copolymers (PLGAs) have been investigated as matrices for releasing contraceptive,[11-14] anaesthetic,[15-17] antinarcotic,[18-20] and antimalarial[21,22] drugs. More recently, PLGAs have found application as controlled release matrices for luteinizing hormone releasing-hormone (LHRH) analogues.[23-26]

Despite the obvious need for such information, the literature fails to provide generally applicable, quantitative correlations between PLGA physical/chemical properties and degradation rates. Much of the current literature is anecdotal: referring to properties of a single drug/polymer combination without extending the information to PLGAs, as a class. Other reports are only semiquantitive and relate polymer behavior to parameters (e.g. drug release, drug excretion, polymer total weight loss, metabolite formation, or radiolabeled polymer recovery) that do not directly depend on the molecular process–ester bond heterolysis—that dictates polymer erosion and molecular weight reduction.

A few communications address some of the molecular parameters that control PLGA degradation, but even these have noticeable shortcomings. Thus, Gilding and Reed[27-29] provided base-line information on chemical and physical properties of various PLGAs including molecular weight determinations by size-exclusion chromatography, SEC. However, these authors failed to quantitatively describe the time dependence of molecular weight changes in hydrolytically degraded polymers. Van Dijk et al.[30] carefully characterized molecular weight distributions of several polylactides and monitored molecular weight changes in partially degraded samples. However, it is not clear whether the methods of this group generally extend to PLGA copolymers. Furthermore, Van Dijk et al. degraded the polylactides under conditions (organic solvent at elevated temperature) not relevant to in vivo degradation. Finally, Pitt and co-workers[31-33] directly compared total weight and molecular weight losses of polylactide (and other polyester) implants in animals and observed a semilogarithmic time dependence for molecular weight loss. Unfortunately Pitt et al. did not attempt to fit their polymer degradation data to kinetic equations that relate molecular weight decreases to the hydrolysis of macromolecular ester linkages.

Considering the foregoing we have undertaken to extend the earlier investigations as follows. We have developed methods to accurately describe the molecular weight distributions of PLGAs as a class and have used these methods to probe PLGA degradation mechanisms in vitro and in vivo. Specifically, for each of ten PLGAs featuring different lactide:glycolide ratios and molecular weights we determined intrinsic viscosities in two solvents and molecular weight distributions by SEC (using the "universal calibration"[34] procedure). For one 50:50 lactide:glycolide copolymer, we incubated cylindrical rods in aqueous buffers at 37 °C and implanted parallel samples subcutaneously in rats. Using the SEC technique, we monitored polymer molecular weight loss versus time and simultaneously determined total weight loss (erosion) rates. The resulting data fit well to a first-order kinetic model that probably will extend to other degradable polyesters. The experiments also provide mechanistic information regarding the degradation process.

Results

Copolymer Characterization. Table I summarizes compositional, viscosity, and molecular weight data for the polymer samples included in the current investigation. Samples P1 through P6 were 60:40 lactide:glycolide copolymers spanning a range of molecular weights and samples P7 through P10 represented copolymers with different lactide:glycolide ratios.

Gilding and Reed[27,28] have shown that PLGAs with 25–65 mol % glycolide monomer are essentially completely amorphous. Except for samples P6 and P7, all of the PLGAS tested have monomer ratios within the 25–65%

* To whom correspondence should be addressed at Travenol Laboratories, 6301 Lincoln, Morton Grove, IL 60053.
† Current address: Varian Associates Instrument Group, Sunnyvale, CA.

0024-9297/87/2220-2398$01.50/0

Table I
Selected Data for PLGA Samples

sample no.	L:G[a]	$[\eta]_{HFIP}$,[b] dL g^{-1}	$[\eta]_{THF}$,[c] dL g^{-1}	$10^{-3}M_n$[d]	$10^{-3}M_w$[d]	M_w/M_n
P1	60:40	2.26	0.944	112	162	1.44
P2	60:40	1.15	0.496	45.2	70.8	1.57
P3	60:40	0.93	e	31.4	49.1	1.56
P4	60:40	0.70	0.358	22.8	40.2	1.76
P5	60:40	0.60	0.300	19.0	31.1	1.63
P6	60:40	0.56	0.293	21.3	34.0	1.59
P7	90:10	0.63	0.312	24.3	39.2	1.61
P8	100:0	0.34	0.148	9.73	14.8	1.52
P9	50:50	0.60	0.233	17.8	28.8	1.61
P10	45:55	0.63	0.232	21.6	31.6	1.47

[a] L:G is the copolymer lactide:glycolide molar ratio. [b] Intrinsic viscosity in hexafluoro-2-propanol solvent at 30 °C. [c] Intrinsic viscosity in tetrahydrofuran solvent at 22.5 °C. [d] Number-average (M_n) and weight-average (M_w) molecular weights from size-exclusion chromatography data in tetrahydrofuran; see text. [e] Not determined.

glycolide range. According to the data of Gilding and Reed the 90:10 and 100:0 PLGAs should exhibit approximately 40 and 50% crystallinity, respectively.

Kricheldorf and co-workers[34] have shown that PLGAs prepared at high temperature with approximately equal monomer ratios exhibit essentially random comonomer distributions. The work of Kricheldorf et al. should extend directly to the 60:40 and 50:50 PLGA samples reported herein. The comonomer distributions are unknown for samples P7 and P8.

Our studies also included monodisperse polystyrene (PS) reference standards. We obtained viscometric and molecular weight distribution data for all samples as described below.

We measured intrinsic viscosities, $[\eta]$, in two solvents, hexafluoro-2-propanol (HFIP) and tetrahydrofuran (THF). The PLGA viscosities in HFIP and THF correlated well, as shown in eq 1 with correlation coefficient, $R = 0.989$.

$$[\eta]_{THF} = (0.032 \pm 0.053) + (0.405 \pm 0.053)[\eta]_{HFIP} \quad (1)$$

From viscosity data (not shown) in THF we calculated the Mark–Houwink relationship for PS standards according to eq 2, where $K = 0.84 \times 10^{-5}$ and $a = 0.745$.

$$[\eta] = KM^a \quad (2)$$

To obtain absolute values for PLGA molecular weight distributions, we used SEC (THF mobile phase) and the "universal calibration" concept.[35] According to this concept, SEC retention volumes (V) correlate with the logarithm of the product $M[\eta]$ for any polymer, regardless of composition. To employ the universal calibration technique, we first directly determined the following values: V_{PS}, $[\eta]_{PS}$, V_{PLGA}, and $[\eta]_{PLGA}$ (where the superscripts PS and PLGA refer to polystyrene and lactide:glycolide copolymer, respectively). The supplier provides accurate M^{PS} values for the standards, but the M^{PLGA} values are unknowns to be determined for use in the "universal calibration" curve. The viscosity-average (M_v) molecular weight for any PLGA sample can be calculated from $[\eta]^{PLGA}$ if the appropriate Mark–Houwink coefficient (K) and exponent (a) values are known. To estimate the PLGA Mark–Houwink constants, we employed an iterative technique,[30,36] wherein we first assumed approximate K and a values for PLGA and then calculated M_v values for a few PLGA samples from SEC elution profiles. We then correlated these estimated M_v values with measured $[\eta]^{PLGA}$ values and calculated new K and a values. The cycle repeated until K and a values converged.

Table II shows the results of the iterative technique applied to PLGA samples P1 and P8. The initial K (= 0.828×10^{-4}) and a (= 0.708) values shown are those reported for polylactide in THF by Van Dijk et al.[30] Table II shows a rapid convergence to constant values, from which we obtain the following Mark–Houwink relationship for PLGA:

$$[\eta]^{PLGA}_{THF} = (1.07 \times 10^{-4})M_v^{0.761} \quad (3)$$

Equations 2 and 3, $[\eta]_{THF}$ data, and SEC elution profiles for PS and PLGA provide the universal calibration curve shown in Figure 1. Least-squares regression of the data

Table II
Iterative[a] Calculations of PLGA Viscosity-Average Molecular Weight (M_v) Values and Mark–Houwink Exponent (a) and Coefficient (K) Values in Tetrahydrofuran

	10^3M_v[b]		Mark–Houwink[c]	
interation no.	PLGA P1[d]	PLGA P2[d]	10^4K	a
1	555	41.7	0.828	0.708
2	260	21.8	0.969	0.738
3	193	16.9	10.3	0.751
4	170	15.2	1.05	0.757
5	162	14.5	1.06	0.759
6	158	14.3	1.06	0.761
7	157	14.2	1.07	0.761
8	156	14.1	1.07	0.761

[a] See text and ref 30 and 37 for a description of the iterative approach. [b] From SEC elution profiles. [c] From the expression $[\eta] = KM^a$. [d] See Table I for polymer composition.

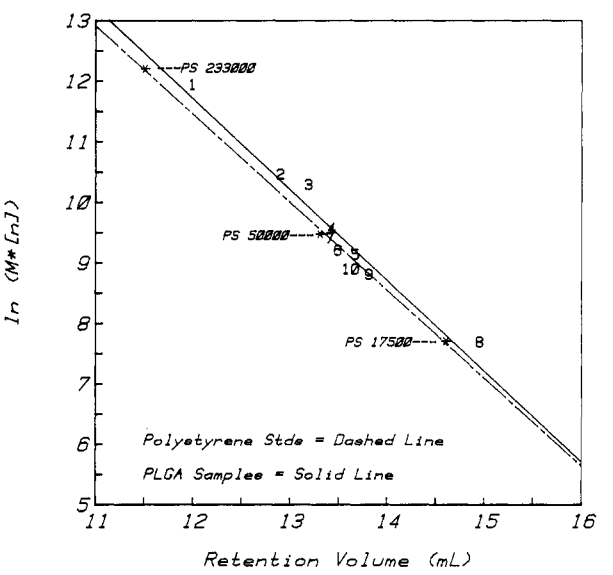

Figure 1. "Universal calibration" curve. Semilogarithmic plot of molecular weight times intrinsic viscosity ($M[\eta]$) versus size-exclusion chromatography retention volume for polystyrene (*–*) and lactide:glycolide copolymers 1–10, corresponding to copolymer sample numbers in Table I.

Table III
Experimental Design for PLGA[a] Degradation Studies[b]

expt no.	1	2	3	4	5
medium	in vivo[c]	H_2O[d]	H_2O[d]	H_2O[d]	H_2O[d]:C_2H_5OH 75:25
pH		4.5	6.0	7.4	7.4

[a] Sample P9, see Table I. [b] All experiments at 37 ± 0.2 °C with 3.2 mm × 7.5 mm cylindrical rods, mean weight = 100 ± 4 mg. [c] Rods implanted subcutaneously in rats. [d] Distilled water plus 0.1 M phosphate buffer adjusted with phosphoric acid to the indicated pH.

in Figure 1 gives the linear calibration expression shown for both PS and PLGA in eq 4 with $R = 0.998$.

$$\ln (M[\eta]) = (29.3 \pm 2.4) - (1.48 \pm 0.18)V \quad (4)$$

Equation 4, $[\eta]^{PLGA}_{THF}$ values, and SEC elution profiles combine to give the number and weight-average molecular weight (M_n and M_w) values shown in Table I. Using these molecular weight values, we have also expressed the SEC calibration curves individually for PLGA and for PS, as shown in eq 5 and 6, respectively, where $R = 0.980$ and $R = 0.999$.

$$\ln (M)^{PLGA} = (21.8 \pm 1.9) - (0.830 \pm 0.14)V^{PLGA} \quad (5)$$

$$\ln (M)^{PS} = (21.8 \pm 0.59) - (0.822 \pm 0.14)V^{PS} \quad (6)$$

Copolymer Degradation Profiles. Table III summarizes the experimental design for our PLGA degradation study. The objective of the study was to provide sufficient data to describe kinetic and mechanistic models for molecular processes accompanying copolymer degradation. Accordingly, we prepared cylindrical rods from PLGA sample P9 (Table I) and incubated them for timed intervals in vitro at 37 °C or in vivo as subcutaneous implants in rats. For each sample, we determined the rod mass and polymer molecular weight distribution as a function of time. Table IV summarizes the mass loss (erosion) data, and Table V provides the molecular weight loss data.

Erosion profiles were very similar in all five experiments, as were molecular weight loss profiles. Figure 2 shows both the erosion and molecular weight loss profiles, respectively, for experiment 3 as an example. Figure 2 qualitatively portrays the three major features of the PLGA degradation curves: (1) mass and molecular weight values were initially unchanged on incubation, (2) after a characteristic in-

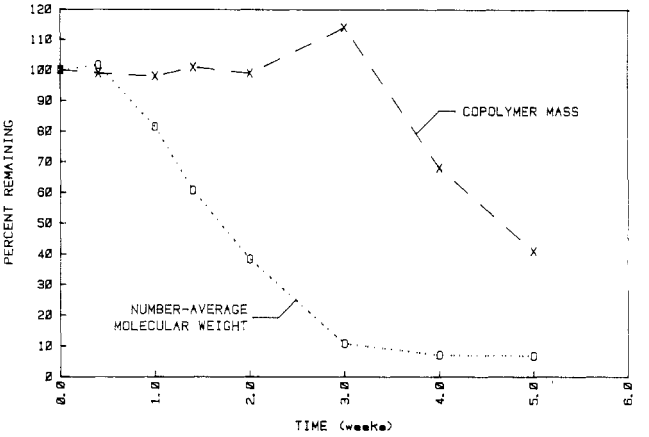

Figure 2. PLGA degradation profile. Percent copolymer mass (×–×) and copolymer number-average molecular weight, M_n (O···O), remaining versus time for experiment 3 (see Table II).

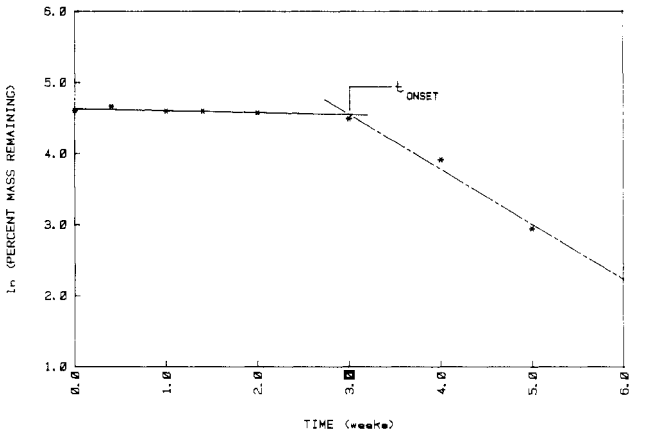

Figure 3. PLGA erosion profile. Semilogarithmic plot of percent copolymer mass remaining versus time for experiment 1 (see Table II).

Table IV
PLGA Erosion at 37 °C in Vivo and in Vitro

	% of initial total mass remaining[b] at t (weeks)							
expt[a]	0	0.40	1.0	1.4	2.0	3.0	4.0	5.0
1	100	96 ± 3	98 ± 2	101 ± 4	99 ± 4	93 ± 10	52 ± 14	11 ± 5
2	100	106 ± 8	99 ± 2	99 ± 2	97 ± 2	89 ± 19	50 ± 40	19 ± 13
3	100	100 ± 2	100 ± 4	100 ± 2	99 ± 2	99 ± 2	66 ± 11	36 ± 5
4	100	99 ± 3	98 ± 2	101 ± 3	99 ± 3	114 ± 6	68 ± 13	41 ± 6
5	100	99 ± 5	101 ± 5	101 ± 5	99 ± 3	c	70 ± 2	57 ± 3

[a] See Table III. [b] Mean of four determinations ± standard deviations. [c] Not determined.

Table V
PLGA Molecular Weight Distributions after Degradation at 37 °C in Aqueous and Aqueous Ethanolic Buffers

	expt[a] no. 1			expt[a] no. 2			expt[a] no. 3			expt[a] no. 4			expt[a] no. 5		
t[c]	$10^{-3}M_n$	$10^{-3}M_w$	M_n/M_w	$10^{-3}M_n$	$10^{-3}M_w$	M_n/M_w	$10^{-3}M_n$	$10^{-3}M_n$	M_n/M_w	$10^{-3}M_n$	$10^{-3}M_w$	M_n/M_w	$10^{-3}M_n$	$10^{-3}M_w$	M_n/M_w
0	16.4	28.2	1.71	17.3	29.2	1.68	17.3	29.2	1.68	17.3	29.2	1.68	17.3	29.2	1.68
0.4	15.7	27.1	1.73	16.4	27.4	1.67	17.5	28.1	1.61	17.6	28.0	1.59	17.5	28.4	1.62
1.0	13.7	23.3	1.70	13.5	23.2	1.73	14.1	24.2	1.71	14.1	24.1	1.71	13.7	24.7	1.80
1.4	11.1	19.3	1.73	10.8	18.6	1.72	11.2	19.0	1.69	10.5	18.9	1.81	11.5	20.4	1.78
2.0	6.20	10.3	1.66	6.72	11.6	1.73	6.92	12.0	1.73	6.65	11.8	1.78	7.04	13.6	1.93
3.0	2.35	3.56	1.52	2.14	3.11	1.45	2.18	3.16	1.45	1.86	2.59	1.39	2.84	4.82	1.70
4.0	1.19	1.51	1.26	1.13	1.35	1.20	1.15	1.37	1.19	1.23	1.58	1.29	1.72	2.24	1.30
5.0	b	b	b	1.06	1.16	1.09	1.10	1.21	1.09	1.18	1.37	1.17	1.53	1.85	1.20

[a] See Table III. All experiments with PLGA sample P9. [b] Unable to assay reliably because of biological tissue contamination and very small sample size. [c] Time (weeks).

Table VI
Kinetic Constants for PLGA Erosion at 37 °C

expt[a] no.	intercept[b] ± SD	k_{obsd} ± SD, week^{-1}	R^b	t_{on},[c] weeks
1	7.89 ± 1.1	1.07 ± 0.28	0.967	3.1
2	6.77 ± 0.19	0.750 ± 0.046	0.998	2.9
3	6.15 ± 0.24	0.508 ± 0.059	0.993	3.1
4	6.27[d]	0.512[d]	d	3.3
5	5.47 ± 0.24	0.300 ± 0.058	0.982	2.8
mean ± SD	6.5 ± 0.9	0.63 ± 0.3		3.0 ± 0.2

[a] See Table III. [b] From least-squares linear regression according to eq 7. [c] From eq 8. [d] Data for t = 4.0 and 5.0 weeks only, no SD or R values derived.

duction period, onset of polymer erosion and molecular weight loss was abrupt, and exponential with time, and (3) erosion onset was considerably slower than onset of molecular weight loss.

Figure 3, a semilogarithmic plot of the mass loss versus time data for experiment 1, clearly demonstrates points 1 and 2 above. The exponential decay in polymer mass and molecular weight suggests pseudo-first-order degradation kinetics. The following section addresses kinetic constants that quantitatively describe the polymer degradation process.

Copolymer Degradation Kinetics. Two kinetic parameters describe the typical erosion profile shown in Figure 3: the onset time (t_{on}) for mass loss and the slope of the line following t_{on}. Because all experiments showed significant erosion only after t = 3.0 weeks, we characterized the slope value according to eq 7, where t = 3.0,

$$\ln (\%\ \text{mass remaining}) = \text{intercept} - k_{obsd}t \quad (7)$$

4.0, and 5.0 weeks only and k_{obsd} is the observed pseudo-first-order rate constant for copolymer erosion. The t_{on} value then derives from intersecting the regression line in eq 7 with the initial mass value, that is

$$t_{on} = [\text{intercept} - \ln (100)]/k_{obsd} \quad (8)$$

Table VI summarizes the polymer erosion kinetic constants for experiments 1 through 5. Considering the limited data points for each experiment, Table VI shows good adherence to eq 7 with high correlation coefficients and with reasonably small relative standard deviations in k_{obsd} and intercept values.

To characterize copolymer molecular weight degradation, we employed the data of Table V and the kinetic model derived by Inokuti[37] for polymers undergoing random chain scission. According to this model, fractional molecular weight averages relate to X, the number of bond cleavages per initial number-average molecule, as shown in eq 9 and 10, where the superscripts t and 0 refer, re-

$$[M_n^t]/[M_n^0] = 1/(1 + X) \quad (9)$$

$$[M_n^t]/[M_n^0] = (2/X)\{1 - X^{-1}[1 - (1 + (X/\beta))^{-\beta}]\} \quad (10)$$

spectively, to degradation reaction time t and time zero. The size distribution parameter, β, is given by eq 11. If

$$\beta = M_w/(M_n - M_w) \quad (11)$$

chain cleavage obeys pseudo-first-order kinetics, the cleavage rate is given by eq 12, where k_d is the copolymer degradation rate constant.

$$\ln (X) = \text{intercept} - k_d t \quad (12)$$

From the data of Table V, plus eq 9 and 12 we have calculated X values and kinetic constants for copolymer degradation in experiments 1 through 5. Table VII summarizes the kinetic constants.

Table VII
Kinetic Constants for PLGA Number-Average Molecular Weight Degradation at 37 °C in Aqueous and Aqueous Ethanolic Buffer

expt[a] no.	intercept[b,c] ± SD	k_d,[b,c] ± SD, week^{-1}	R
1	−2.66 ± 0.41	1.39 ± 0.16	0.979
2	−2.38 ± 0.31	1.33 ± 0.12	0.988
3	−2.57 ± 0.36	1.38 ± 0.14	0.984
4	−2.44 ± 0.47	1.36 ± 0.19	0.973
5	−2.30 ± 0.34	1.20 ± 0.14	0.981
mean	−2.42 ± 0.11	1.32 ± 0.080	

[a] See Table III. [b] From linear least-squares regression according to eq 12. [c] For 0.40 < t < 5.0 weeks, see text.

All experiments showed good adherence to eq 12 for time points less than 5.0 week. At the 5-week points, M_n values were quite low and possibly outside the linear range of the SEC calibration plot (eq 5). Thus we excluded the 5-week points from the regression calculation. Similarly, at t = 0 and 0.40 week, polymer molecular weight loss had not begun and we also excluded these time points from the calculations.

Discussion

Polymer Characterization. Our Mark–Houwink constants for PS (eq 2) and PLGA (eq 3) in THF compare very favorably with literature values. For PS, Spatorico and Coulter[36] prefer $K = 1.11 \times 10^{-4}$ and $a = 0.725$, and for related the aliphatic polyester, poly(caprolactone), Schindler et al.[38] report $K = 1.4 \times 10^{-4}$ and $a = 0.786$. For the PLGAs reported here, THF and HFIP serve equally well as solvents for intrinsic viscosity measurements. However THF is advantageous with respect to cost and safety. Equation 1 relates viscosity data for THF and HFIP and permits calculations for one solvent if $[\eta]$ values for the other solvent are known.

The SEC method reported here accurately measures PLGA molecular weight distributions with a linear range of approximately (0.5–300) × 10^3 molecular weight. We also have confirmed (Figure 1) that the "universal calibration" concept for SEC extends to PLGA copolymers, and this now permits extension of the SEC method to various PLGAs currently under investigation in our laboratory and elsewhere. The PLGA SEC calibration line shown in eq 5 allows direct calculation of molecular weight distributions without resorting to further intrinsic viscosity measurements.

Copolymer Degradation Profiles. Copolymer erosion and molecular weight profiles (e.g. see Figure 2) both show an induction period where no time-dependent changes occur. For molecular weight loss, the induction period is short (approximately 3 days) and presumably reflects the interval required for water to completely permeate the polymer structure. For the erosion process, the induction period is longer (approximately 3 weeks) and has mechanistic significance: because onset of erosion lags behind molecular weight loss, PLGA hydrolysis must proceed throughout the bulk of the polymer structure. An alternative mechanism, surface layer hydrolysis and erosion, requires that erosion precede molecular weight degradation of retrieved polymer samples.

Reed and Gilding[28] inferred the bulk hydrolysis mechanism for a 50:50 PLGA sample incubated at pH 7, 37 °C, but their data (Figure 6 of ref 28) do not reveal the differential erosion versus molecular weight loss rates clearly seen in Figure 2 of this report. Schindler et al.,[31] Pitt et al.,[33] and Benagiano and Gabelnick[39] did demonstrate bulk versus surface erosion mechanisms for polylactide and

other aliphatic polyesters. Thus, our results confirm earlier work and suggest that bulk erosion is rather a general mechanism for polyester hydrolysis.

Copolymer Degradation Kinetics. Tables V and VI show an interesting independence of erosion and molecular weight loss rates on reaction medium under the conditions studied. Erosion rate constant (k_{obsd}) values were constant to within a factor of approximately 2, and t_{on} values were constant to within ±15% for all give experiments. Similarly, molecular weight loss rate constant (k_d) values were constant to within ±6%.

In a practical context, the independence of degradation kinetic constants on the reaction medium suggests that in vitro studies under any of the conditions reported here should accurately model copolymer behavior in vivo. Mechanistically, the insensitivity of copolymer degradation rate to reaction medium pH suggests an acidic microenvironment resulting from carboxylic acid formation subsequent to polyester bond cleavage. The suggestion of an acidic microenvironment is consistent with previous literature references to pH-independent polyester hydrolysis reaction[28,31,33] and raises an interesting question with respect to drug delivery devices. In cases where PLGA degradation precedes drug release, will drug degradation suffer acid catalysis and concomitant bioavailability decrease? For acid-labile drugs, the intervention of autocatalytic drug decomposition seems probable.

The PLGA hydrolysis kinetics provide additional insight into the characteristics of copolymers undergoing degradation and erosion. For experiments 1 through 5, PLGA erosion onset began at 3.1 weeks. From Table V, PLGA molecular weights at t = 3.0 weeks averaged M_n = 2300 ± 280, reflecting the molecular weight at which this PLGA gains appreciable water solubility. It is also interesting to compare the number of bond cleavages per initial number-average molecule required to convert this specific PLGA to a water-soluble material. For $M_n = 18.7 \times 10^3$, the 50:50 lactide:glycolide copolymer (sample P9, Table I) has degree of polymerization = M_n/(mean monomer molecular weight) = 231. At t = 3.0 weeks, for the same polymer in experiments 1 through 4, the number of bond cleavages (eq 9) averaged $X = 6.7 \pm 1.2$. Thus for sample P9, only 100(6.7/231) = 3% of the ester bonds must cleave before erosion ensues.

Conclusions

We have used viscometry and size-exclusion chromatography to characterize lactide:glycolide copolymers (PLGAs). These are not new methods, but it is significant that we have demonstrated method validity for copolymers covering a range of lactide:glycolide ratios. The Mark–Houwink relationships and SEC calibrations reported here should conveniently extend to other laboratories choosing to adopt our methods and conditions.

We find the SEC method to be a particularly useful probe for monitoring molecular processes accompanying polyester hydrolysis. For the specific case of a 50:50 lactide:glycolide copolymer we have demonstrated the following: polymer erosion proceeds via internal, not surface, hydrolysis and dissolution, polymer erosion and molecular weight degradation at 37 °C, pH 4.5, to 7.4 in vitro accurately model the processes in vivo, and PLGA degradation adheres to pseudo-first-order kinetics governed by the rate of macromolecular ester bond cleavages.

As a whole, our findings emphasize some perspectives on the design and interpretation of PLGA degradation studies (past and future). First, we emphasize that PLGA erosion initially does not parallel macromolecular bond heterolysis. Therefore directly relating polymer erosion to parameters (e.g. lactide:glycolide ratio) that control the bond cleavage kinetics is potentially misleading. Secondly, the (presumably acidic) microenvironment interior to hydrolyzing PLGAs could potentially accelerate the degradation of drugs or other materials dispersed throughout the PLGA matrix material. Finally, eq 9 and 10 which relate polymer molecular weight changes to the number of ester bond cleavages per initial number-average molecule place our understanding of the degradation process at the molecular level. This perspective is important because it should generally extend to all degradable polyesters regardless of monomer composition or initial molecular weight distribution.

Experimental Details

Materials. Hexafluoro-2-propanol (reagent grade, Fisher Scientific), tetrahydrofuran (distilled in glass, Burdick and Jackson Lab, Inc.), *d,l*-lactic acid (USP grade, Monsanto Co.), glycolic acid (technical grade, Ashland Chemical Co.), and polystyrene narrow molecular weight distribution reference standards (Supelco, Inc.) were purchased from the indicated suppliers.

The poly(lactide-*co*-glycolide) samples were prepared by charging glycolide and lactide monomers into 18-mm diameter test tubes and heating at 130 °C under inert atmosphere. Reagent grade 1-dodecanol was added as chain terminator and 0.033% stannous octoate was the catalyst. The polymers were purified by dissolving the crude reaction mixture in methylene chloride, followed by filtration through a cotton plug and precipitation from methanol. The general procedure was as described by Beck et al.[13]

The polymers were extruded as 3.2-mm diameter cylindrical rods and cut to 7.4-mm lengths weighing approximately 100 mg. Each sample was sterilized by γ-irradiation (1.25 Mrad). The in vivo studies employed female Sprague–Dawley derived rats from the Syntex animal colony.

Apparatus. Relative viscosities were measured in a No. 75 Cannon-Fenske viscometer (American Scientific Products). The size-exclusion chromatography system included the following components: pump (Model M6000B, Waters Assoc.), autosampler (Model 710B, Waters Assoc.), two columns (μStyragel, 10^3 Å and 10^4 Å, 2 × 300 × 7.8 mm, Waters Assoc.), detector (Spectromonitor III, Laboratory Data Control), printer/plotter (Model 730, Waters Assoc.), data system (Model 4000, Spectra Physics).

Temperature control for in vitro experiments was maintained at 37 ± 0.2 °C with a water bath and circulating immersion heater (Thermomix, B. Braun). Linear regression analyses are reported throughout with 95% confidence intervals unless otherwise indicated.

Viscometric Methods. Measurements in THF were performed at 4 mg/mL under ambient (22.5 °C) conditions. Measurements in HFIP were at 5 mg/mL and 30 °C. Control experiments demonstrated adherence of relative viscosity versus polymer concentration data to the Huggins equation.[40] Subsequent intrinsic viscosity calculations used the single concentration technique of Solomon and Ciuta.[41]

Size-Exclusion Chromatography Methods. The chromatographic operating conditions were as follows: mobile phase = 100% THF; sample concentration = 1 mg/mL in THF; injection volume = 0.20 mL; flow = 1 mL/min; detection = 230 nm × 0.1 AUFS. Polystyrene and PLGA calibration plots were empirically corrected for unsymmetrical (λ) and symmetrical (δ) band broadening by using λ = 1.04 and δ = 0.192 and the equations of Yau et al.[42]

Copolymer Degradation Studies. The in vivo samples were implanted subcutaneously at the nape of the neck, explanted at timed intervals, and blotted dry. The in vitro samples were contained individually in 4-mL vials filled to 3 mL with the appropriate reaction medium and similarly withdrawn for study. All retrieved samples were dried to constant weight in vacuo over P_2O_5 and stored over P_2O_5 before analysis.

Acknowledgment. We gratefully acknowledge the contributions of Dr. G. Wayne Whitehead (Syntex Chemicals) in preparing the polymers and of Dr. Thomas Nem-

zek (Shell Development) in providing helpful suggestions on molecular weight characterization methods.

Registry No. PLGA, 59199-59-6; hexafluoro-2-propanol, 920-66-1; tetrahydrofuran, 109-99-9.

References and Notes

(1) Hafez, E. S.; van Os, W. A. A., Eds. *Biodegradables and Deliver Systems for Contraception*; G. K. Hall Medical Publishers: Boston, 1930.
(2) Das, K. G., Ed. *Controlled-Release Technology Bioengineering Aspects*; Wiley-Interscience: New York, 1983.
(3) Kopece, K. J.; Ulbrich, K. *Prog. Polym. Sci.* **1983**, *9*, 1.
(4) Wood, D. A. *Int. J. Pharm.* **1980**, *7*, 1.
(5) Robinson, J. R., Ed. *Sustained and Controlled Release Drug Delivery Systems*; Marcell Dekker: New York, 1978.
(6) Benagiano, G.; Gabelnick, H. L. *J. Steroid Biochem.* **1979**, *11*, 449.
(7) *Polymer Delivery Systems, Midland Macromolecular Institute Monograph*; Gordon and Breach: New York, 1978; Vol. 5, pp 1–23.
(8) Paul, D. R.; Harris, F. W., Eds. *Controlled*-Release *Polymeric Formulations*; American Chemical Society: Washington, D.C., 1976.
(9) Gabelnick, H. L., Ed. *Drug Delivery Systems*, USDHEW Pub. No. (NH)77-1238; U.S. Department Health Education and Welfare: Washington, D.C., 1977.
(10) Gregoriades, G., Ed. *Drug Carriers in Biology and Medicine*; Academic: New York, 1979.
(11) Jackanicz, T. M.; Nash, H. A.; Wise, D. L.; Gregory, J. B. *Contraception* **1973**, *8*, 227.
(12) Wise, D. L.; Fellman, T. D.; Sanderson, J. E.; Wentworth, R. L. In *Drug Carriers in Biology and Medicine*; Gregoriades, G., Ed.; Academic: New York, 1979; pp 237–270.
(13) Beck, L. R.; Pope, V. Z.; Flowers, C. E.; Cowsar, D. R.; Tice, T. R.; Lewis, D. H.; Dunn, R. L.; Moore, A. B.; Gilley, R. M. *Biol. Reprod.* **1983**, *28*, 186.
(14) Wise, D. L.; Gregory, J. B.; Newborne, P. M.; Bartholow, L. C.; Standbury, J. B. In *Polymeric Delivery Systems*; Gordon and Breach: New York, 1978; pp 121–138.
(15) Wakiyama, N.; Juni, K.; Nakano, M. *Chem. Pharm. Bull.* **1982**, *30*, 2621.
(16) Wakiyama, N.; Juni, K.; Nakano, M. *Chem. Pharm. Bull.* **1982**, *30*, 3363.
(17) Wakiyama, N.; Juni, K.; Nakano, M. *Chem. Pharm. Bull.* **1982**, *30*, 3719.
(18) Schwope, A. D.; Wise, D. L.; Hawes, J. F. *Life Sci.* **1975**, *17*, 1877.
(19) Schwope, A. D.; Wise, D. L.; Harrigan, S.; McCarthy, D. S.; Hawes, J. F. In *Polymeric Delivery Systems*; Gordon and Breach: New York (1977).
(20) Harrigan, S. E.; McCarthy, D. A.; Reunning, R.; Thies, C. In *Polymeric Delivery Systems*; Gordon and Breach: New York, 1978; pp 91–100.
(21) Wise, D. S.; McCormick, G. J.; Willet, G. P.; Anderson, L. C.; Hawes, J. F. *J. Pharm. Pharmacol.* **1978**, *30*, 686.
(22) Wise, D. L.; McCormick, G. J.; Willet, G. P. *Life Sci.* **1976**, *19*, 867.
(23) Sanders, L. M.; Kent, J. S.; McRae, G. I.; Vickery, B. H.; Tice, T. R.; Lewis, D. H. *Arch. Androl.* **1982**, *9*, 91.
(24) Ahmed, S. R.; Grant, J.; Shalet, S. M.; Howell, A.; Chowdhury, S. D.; Weatherson, T.; Blacklock, N. J. *Br. Med. J.* **1985**, *290*(6463), 185.
(25) Mason-Garcia, M.; Vigh, S.; Comaru-Schally, A. M.; Redding, T. W.; Somogyvari-Vigh, A.; Horvath, J.; Schally, A. V. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1547.
(26) Asano, M.; Yoshida, M.; Kaetsu, I.; Imai, K.; Mashino, T.; Yuasa, H.; Yamanaka, H.; Suzuki, K.; Yamazaki, I. *Makromol. Chem., Rapid Commun.* **1985**, *6*, 509.
(27) Gilding, D. K.; Reed, A. M. *Polymer* **1979**, *20*, 1459.
(28) Reed, A. M.; Gilding, D. K. *Polymer* **1981**, *22*, 494.
(29) Gilding, D. K., Reed, A. M.; Askill, I. N. *Polymer* **1981**, *22*, 505.
(30) Van Dijk, J. A. P. P.; Smito, J. A. M.; Kohn, F. E.; Feijen, J. *J. Polym. Sci.* **1983**, *21*, 197.
(31) Schindler, A.; Jeffcoat, R.; Kimmel, G. L.; Wall, M. E.; Zweidinger, R. In *Contemporary Topics in Polymer Science*; Pearce, E., Schaefegen, J., Eds.; Plenum: New York, 1977; Vol. 2, pp 251–289.
(32) Pitt, C.; Christensen, D.; Jeffocat, R.; Kimmel, G. L.; Schindler, A.; Wall, M. E.; Zweidinger, R. A. In *Drug Delivery Systems*, DHEW Pub. No. (NIH) 77-1238; U.S. Department Health, Education Welfare: Washington, D.C., 1977.
(33) Pitt, C. G.; Chasalow, F. I.; Hibionada, Y. M.; Klimas, D. M.; Schindler, A. *J. Appl. Polym. Sci.* **1981**, *26*, 3779.
(34) Kricheldorf, H. R.; Jonte, J.; Berl, M. *Macromol. Chem. (London), Suppl. 12* **1985**, 25.
(35) Grubisic, Z.; Rempp, P.; Benoit, H. *Polym. Lett.* **1967**, *5*, 753.
(36) Spatorico, A. L.; Coulter, B. *J. Polym. Sci., Polym. Phys. Ed.* **1973**, *11*, 1139.
(37) Inokuti, M. *J. Chem. Phys.* **1963**, *38*, 1174.
(38) Schindler, A.; Hibionada, Y. M.; Pitt, C. G. *J. Polym. Sci., Polym. Chem. Ed.* **1982**, *20*, 319.
(39) Benagiano, G.; Gabelnick, H. L. *J. Steroid Biochem.* **1979**, *11*, 449.
(40) Huggins, M. L. *J. Am. Chem. Soc.* **1942**, *64*, 2716.
(41) Solomon, O. F.; Ciuta, I. Z. *J. Appl. Polym. Sci.* **1962**, *6*, 683.
(42) Yau, W. W.; Kirkland, J. J.; Bly, D. D. *Modern Size-Exclusion Chromatography*; Wiley-Interscience: New York, 1978.

Vibrational Circular Dichroism of Polypeptides. 11. Conformation of Poly(L-Z-lysine-L-Z-lysine-L-1-pyrenylalanine) and Poly(L-Z-lysine-L-Z-lysine-L-1-naphthylalanine) in Solution

S. C. Yasui and T. A. Keiderling*

Department of Chemistry, University of Illinois at Chicago, Chicago, Illinois 60680

Masahiko Sisido

Research Center for Medical Polymers and Biomaterials, Kyoto University, Kyoto 606, Japan. *Received February 25, 1987*

ABSTRACT: The vibrational circular dichroism of the title compounds in DMSO has been measured in the amide A, I, and II regions. The data are shown in both cases to be consistent with a right-handed α-helical structure being the dominant conformation. VCD avoids the interference from transitions on the aromatic substituent that makes such determinations very difficult to realize with electronic CD data alone. The measured $\Delta A/A$ values in the VCD are somewhat lower than have been seen for other known right-handed α-helices which may be attributable in part to solvent effects.

Introduction

Recently it has become possible to use vibrational circular dichroism (VCD) as an additional spectroscopic probe of polypeptide secondary structure.[1] In particular, a clear pattern has been derived for the VCD of the right-handed α-helix[2] which has been shown to be distinguishable from that of the random coil (with or without local order) and the antiparallel β-sheet.[3] Additionally, Yasui et al. have shown the 3_{10}-helix to have a VCD spectrum distinguishable from that of the α-helix.[4] This latter result implies a greater specificity in VCD for slightly differing conformations than is available with the more

0024-9297/87/2220-2403$01.50/0